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Relationship Between Eosinopenic Response to Cold Application and the Adrenal Gland in the Dog

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The dogs were immersed into the ice water for periods of 18–55 minutes. Following withdrawal from the ice water bath, peripheral blood was sampled at an interval of 30 minutes for 5–8 hours and was used for the eosinophil counts. Bilateral adrenalectomy was performed on dogs without anesthesia. The right adrenal gland had been extirpated about 4–20 days before and the left gland at the day of observation. Cold application induced a profound fall in the number of the circulating eosinophils of the intact and of the chronically adrenal demedullated dog, its lowest level being noted within approximately 2–4 hours after the withdrawal. However, the adrenalectomized dog exhibited no or only a slight and transient fall in the eosinophil levels following cold application. Our results seem to warrant the conclusion that the eosinopenic response of the intact dog to cold application is, in the major part, due to the increased adrenal cortical activity.

A marked decline in the circulating eosinophils induced by stress has long been regarded as a result of the adrenal cortical response to corticotropin. However, there is a considerable number of reports in this respect, suggesting that the eosinopenic response to stress is not specific for adrenal steroids. In the cortisone-treated subject, MUEHRCKE, STAPLE and KARK[5] observed that an injection of adrenaline induced a definite decline in the eosinophils, despite complete interruption of the pituitary adrenal system. Other investigators noted that a profound eosinopenia occurred by treatment of the bilaterally adrenalectomized subject with adrenaline,[4] plasma protein,[1] bacterial pyrogen[7] and trauma,[2] etc. Evidence of SPEIRS and MEYER[9] in the mouse would seem to indicate that the eosinophil fall of the adrenalectomized animal in response to stress or to adrenaline depends upon the release of hormones from the accessory cortices.

Of interest is the finding of SWINGLE *et al.*[12][13] that the adrenalectomized dogs maintained on DOC exhibited a marked eosinopenia under the condition evoking unusual muscular activity as a stress. They state that the stress-induced eosinopenia in the adrenalectomized

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animal is due to factors other than hormones of the adrenal cortex, probably due to histamine released from the tissue.

The experiments to be described here have been designed to investigate whether cold application, which induces hypothermia, affects the circulating eosinophils of the intact, the adrenal demedullated and the adrenalectomized dog.

METHODS

Mongrel dogs ranging in weight from 6.2 – 14.1 kg were used in this investigation. Experiments were performed on intact, adrenal demedullated and adrenalectomized dogs. Adrenalectomy was achieved on the dogs, whose dorsal spinal roots ($T_{11}-L_3$), through which the sensory nerve fibers from the lumber area run, were cut under ether anesthesia about 2–10 months before experiments. In these dogs, the removal of the right adrenal gland was performed through the lumbar route about 4–20 days before and that of the left gland at the time of observation. The operative procedures described above for extirpating the adrenals were carried out without anesthetic or pain.

At the day of observation, the animals were immersed into ice water for periods of 18–55 minutes. Intermittent rectal temperature measurements were made during the period of cold application. Immediately after the rectal temperature had been reduced to approximately 30°C, the animals were taken out from the ice water bath. This required 19–50 minutes. For studying alteration in the number of the circulating eosinophils and of leucocytes, blood sampling was made several times before cold application and also at 30-minute intervals for 5–8 hours from the time of the withdrawal. Blood was collected from the saphenous vein.

The eosinophil cell counts were carried out by use of the method of SPEIRS and MEYER[8] using the Fuchs-Rosenthal counting chamber. Venous blood was drawn into a white cell count pipet up to 0.5 mark, as in performing the usal leucocyte counts, and then diluted with 2% eosin-acetone mixture. The pipet was shaken throughly for thirty seconds. The Fuchs-Rosenthal counting chamber was filled immediately, and the eosinophils were counted after five minutes or more. Diluent consists of :

Eosin, aqueous, 2 percent	5 cc
Acetone	5 cc
Distilled water	90 cc

All eosinophil and leucocyte values are expressed as the percent difference between the count before and that after cold application.

RESULTS

Cold Application on the Intact Dogs

In these experiments, 5 intact dogs were used. The rectal temperatures of these animals varied between 37.2 and 38.5°C before cold application. While as a result of treatment with the ice water, a marked fall in rectal temperature occurred, its lowest level (22.2–31.0°C) being noted 3–6 minutes after the withdrawal. Its temperatures returned almost to basal level by the 5th–6th hour. There was, in addition, an increase in heart rate and a slight decrease in respiration following withdrawal from the ice water bath.

The results are illustrated in Figs. 1–4. Four out of 5 animals in this group had a striking fall in the number of circulating eosinophils, the maximum decrease in eosinophils occurred within approximately 2–4 hours after the withdrawal. The level of eosinophils decreased 79% from the control in Fig. 1, 88% in Fig. 2, 74% in Fig. 3 and 88% in Fig. 4. Concomitantly with eosinopenia, these animals exhibited the maximum rise in the leucocyte levels within 2–4 hours.

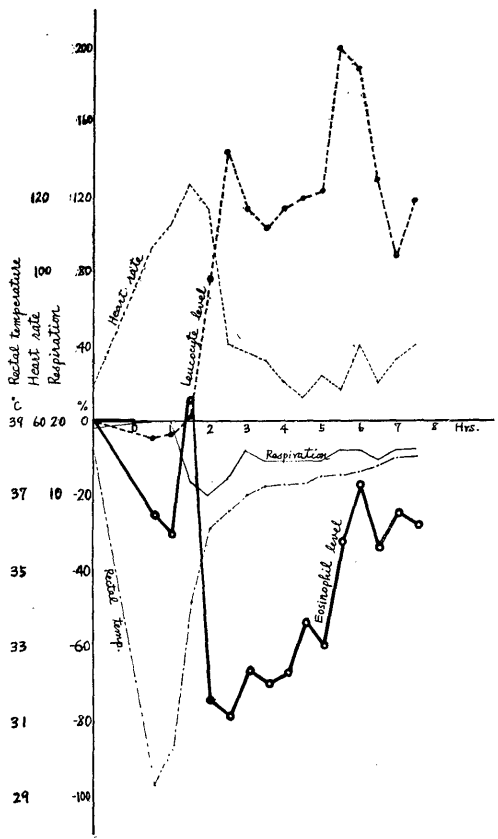

Fig. 1. Chart showing the eosinophil changes after cold application in the intact female dog, 10.3 kg in weight. Room temperature was between 15.5 and 16°C.

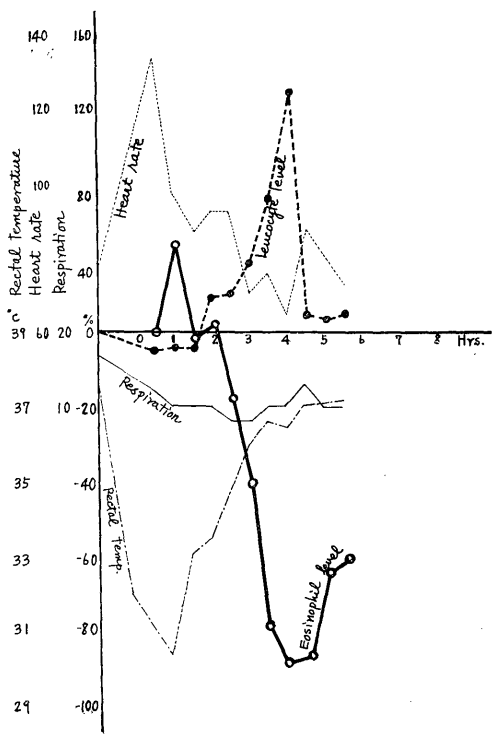

Fig. 2. Intact female dog, 9.3 kg in weight. Room temperature was between 15 and 17°C.

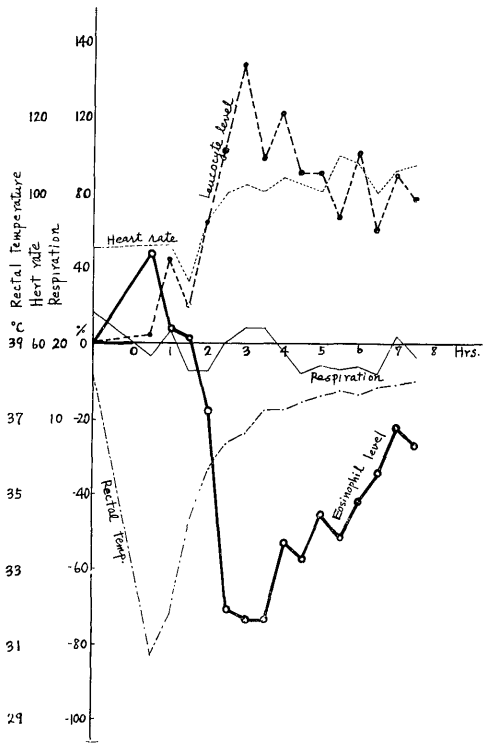

Fig. 3. Intact female dog, 11.1 kg in weight. Room temperature was 14–14.5°C.

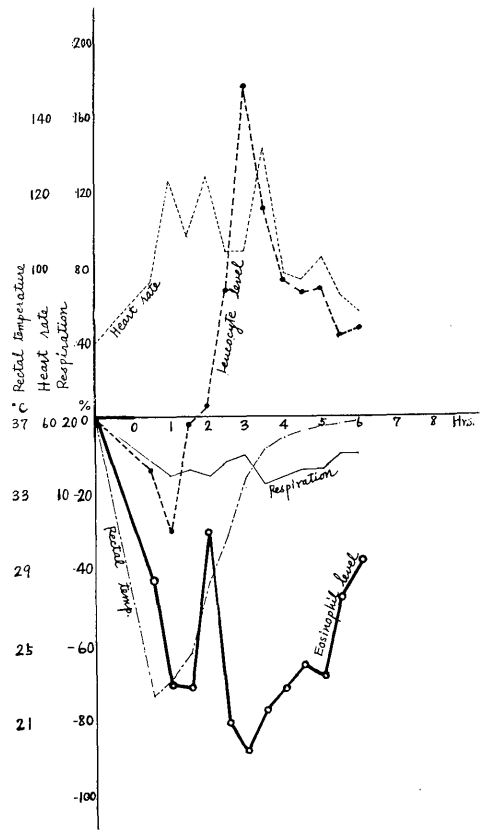

Fig. 4. Intact male dog, 6.2 kg, in weight. Room temperature was between 21 and 22°C.

It was also observed that the time course of the leucocyte increase was related extremely well to that of eosinophil decrease. In the remaining one animal, there was no response to the cold application, in spite of a marked fall of the rectal temperature.

Cold Application on the Adrenalectomized Dogs

In this series, experiments were performed on dogs, whose thoracic (11) – lumbar (3) dorsal spinal roots corresponding to the surgical operation area were cut about 2–10 months. The right adrenal gland was removed about 4–20 days before and the left gland at about 2 hours before observation without anesthesia.

Prior to cold application, the rectal temperature of these animals were between 38.7 and 40.5°C. Cold application resulted in a remarkable fall in the rectal temperature, which showed a maximum lowering such as 24.0–27.4°C at 33–45 minutes after withdrawal from ice water. Afterward the rectal temperature failed to resume its basal level within 6 hours of the observation.

The circulating eosinophils in 2 out of 3 animals showed only a

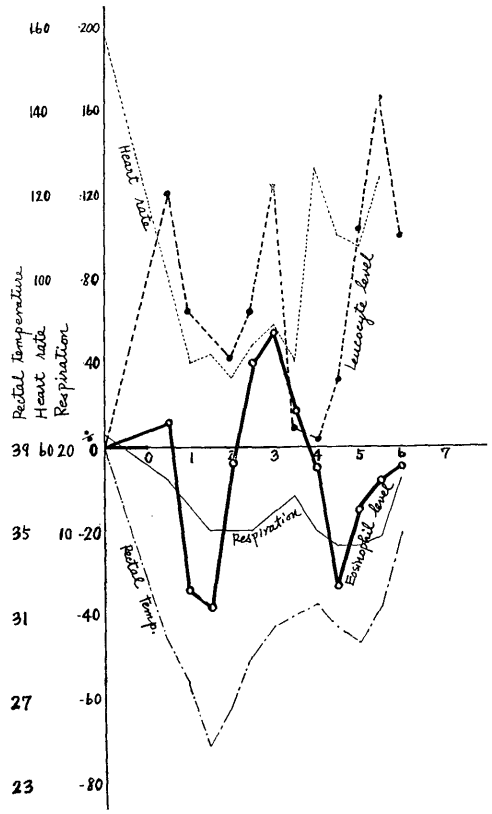

Fig. 5. Bilaterally adrenalectomized female dog, 11.6 kg in weight. Room temperature varied between 16 and 18°C.

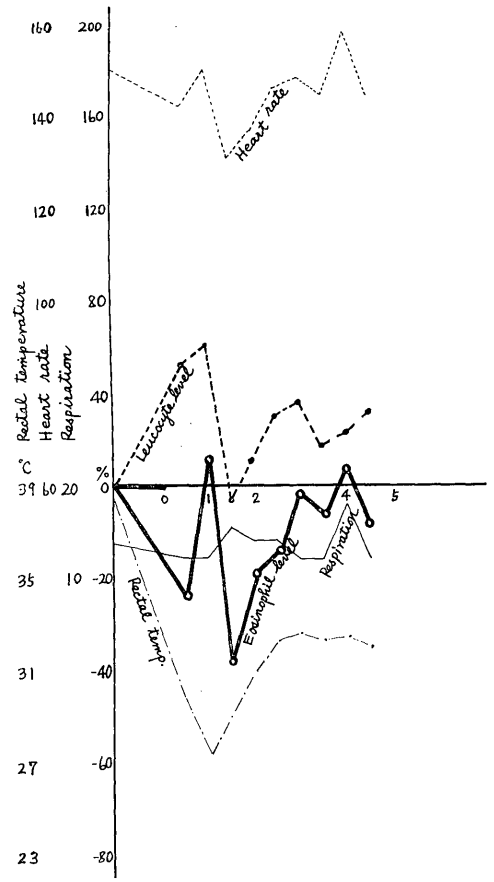

Fig. 6. Bilaterally adrenalectomized male dog, 14.1 kg in weight. Room temperature was between 14.5 and 16°C.

38% fall within 60 minutes following the withdrawal. The response was such a transitory one that the eosinophil levels returned to almost basal levels within 30 minutes after reaching a maximum decline. In these animals, a slight leucocytosis developed with some fluctuation from just after the withdrawal to the end of observation. The remaining one animal did not respond to cold application on the variation of circulating eosinophils, the level being relatively constant throughout the observation. However, concerning the leucocyte changes, there was a considerable decline in the number of leucocytes. The lowest decline appeared mostly within 60 minutes after the end of cold application, a 78% fall being observed.

Cold Application on the Adrenal Demedullated Dog

Only one animal was tested in this experiment. The left adrenal medulla was removed by the lumber route 15 days prior to and the

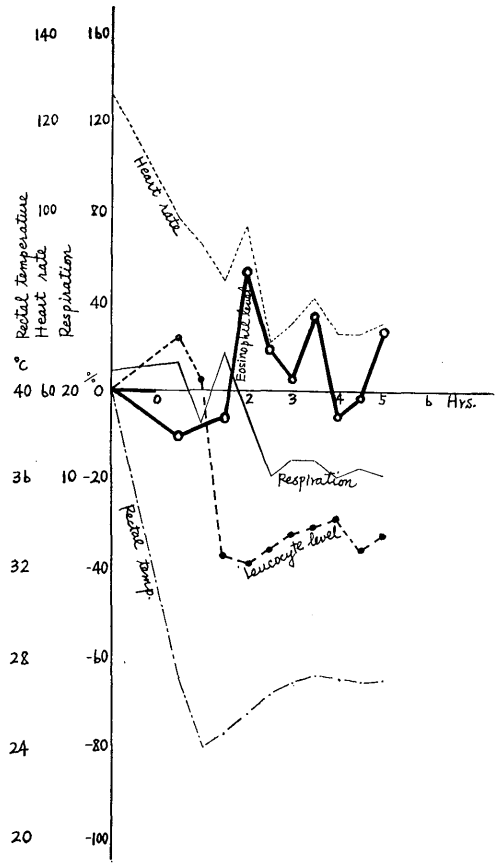

Fig. 7. Bilaterally adrenalectomized male dog, 6.4 kg in weight. Room temperature was 23.5—24.5°C.

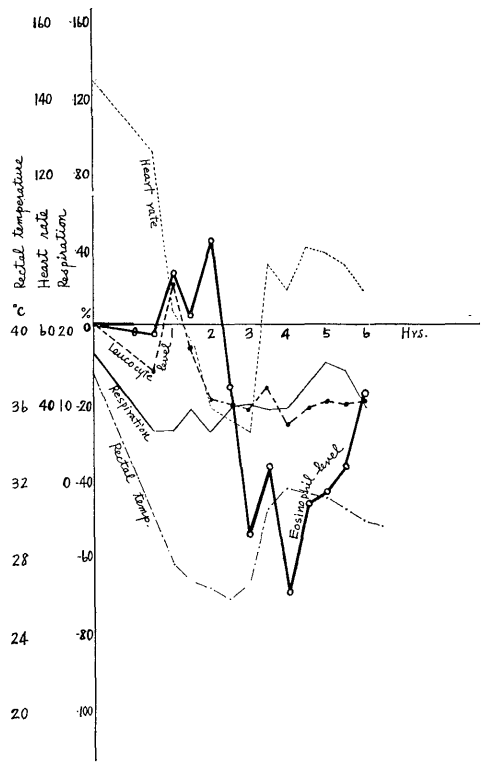

Fig. 8. Bilaterally adrenal demedullated female dog, 5.3 kg in weight. Room temperature was 18°C.

right one 9 days before the observation.

The results are shown in Fig. 8. By immersing into the ice water bath for 19 minutes, the rectal temperature fell markedly from 37.4°C, the lowest level such as 25.7°C appeared within about 2 hours after the withdrawal.

Cold application produced a marked eosinopenia in this demedullated animal. The animal exhibited a maximum fall at 3.5 hours following the withdrawal, causing a 69% decline from the basal. On the other hand, the cold application produced only a slight fall in leucocyte level.

DISCUSSION

In our experiments, a marked fall in the number of the circulating eosinophils could be induced by cold application in the intact dog.

However, the adrenalectomized dog exhibited no or only a slight decrease, which is of a transient nature, in the eosinophil levels in response to cold appliation. In this respect, it has previously been demonstrated by RECANT, HUME, FORSHAM and THORN[6] that bilateral adrenalectomy interfered with the fall in the eosinophil levels resulting from stress. This was further evidenced by numerous experiments of the others. These findings lead to the idea that the adrenal gland plays an important rôle in the eosinopenic response to stress.

On the question of how cold application used in present investigation affects the adrenal medullary secretion, an increase in adrenaline secretion of the adrenal after cold application has already been reported by WADA, SEO and ABE.[15] The effect has been demonstrated by direct measurement of the adrenaline in adrenal venous blood and the oversecretion extended over about 100 minutes after the start of cold application. In their experiments, almost the same procedure as that reported here was employed. In our experiment to elucidate the question as to whether the adrenal medulla is concerned with the mechanism of eosinopenic response to cold application, the adrenal demedullated animal showed a profound fall in the circulating eosinophils, the magnitude of decrease being practically the same as that of the animal with intact adrenal gland. RECANT *et al.*[6] provided evidence that adrenaline has no eosinopenic action in adrenalectomized rats and dogs. On the other hand, a number of other observations[3)4)11)] show that administration of adrenaline lead to an appreciable fall in the circulating eosinophils. Demonstrating that in intact mice pre-treated with adrenaline the injection of ACTH induced no decrease in the circulating eosinophils, VERSCHOOF[14] indicates that adrenaline has an inhibitory effect on the adrenal cortex. Considering from our results, it seems unlikely that the adrenal medullary hormones contribute directly to the major part of eosinopenic mechanism in response to cold application in the intact animal.

Some years ago, using the almost same procedure as that described here, when the effect of hypothermia on the secretion of 17-hydroxycorticosteroids of the adrenal gland in unanesthetized dogs was investigated in our laboratory,[10] an increase in 17-hydroxycorticosteroid secretion was invariably noted. These findings would seem to harmonize with the results of the present investigation that the eosinopenic response of the dog with the intact adrenal to cold application is due to, in the major part, the release of adrenal cortical hormones.

On the other hand, SWINGLE *et al.*[12] have provided evidence on a marked eosinopenia induced by severe stress (violent muscular exertion and electrical stimulation) in the adrenalectomized dogs maintained on

DOC and suggested that the fall in the circulating eosinophils of the adrenalectomized dog following stress is due to factors other than adrenal cortical hormones. They also state that, as a stress to induce eosinopenia in the adrenalectomized dog, it requires more stronger stress than that for the intact animal. This is, however, not the case in our experiments, despite our experimental procedure is so severe that the rectal temperature falls to about 24.0–27.4°C. SWINGLE *et al.*[13] found, in a further experiment, that a decrease in eosinophils after injections of histamine, pituitrin, adrenaline, regitine and No. 48–80 was abolished by pre-treatment with pyribenzamine, the antihistaminic agent, which was ineffective in preventing the eosinopenia occurred following injection of cortisone. According to them, the eosinopenic agent is to be histamine. In spite of these observations, they did not give evidence of an inhibitory effect of pyribenzamine on the eosinopenic response to violent muscular exertion or to electrical stimulation in the adrenalctomized dog. Hereupon, in their experiments, it should not be concluded that in the adrenalectomized dog a sharp decrease in the eosinophils induced by stress is, in the major part, due to histamine releasing action of stress or of drugs.

Only a slight decrease in the circulating eosinophils of the adrenalectomized dog following cold application seen in our experiments might be due to factors other than hormones of adrenal cortex, for example histamine released from the tissue and noradrenaline derived from the endings of adrenergic fibers, etc. in response to cold application. However, we have not at present any conclusive evidence in this point.

In any way, by means of cold application we have employed, we failed to confirm the finding of SWINGLE *et al.* Our experimental results lead to the view that the eosinopenic response to cold application is related, in the major part, to the adrenal cortical response.

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